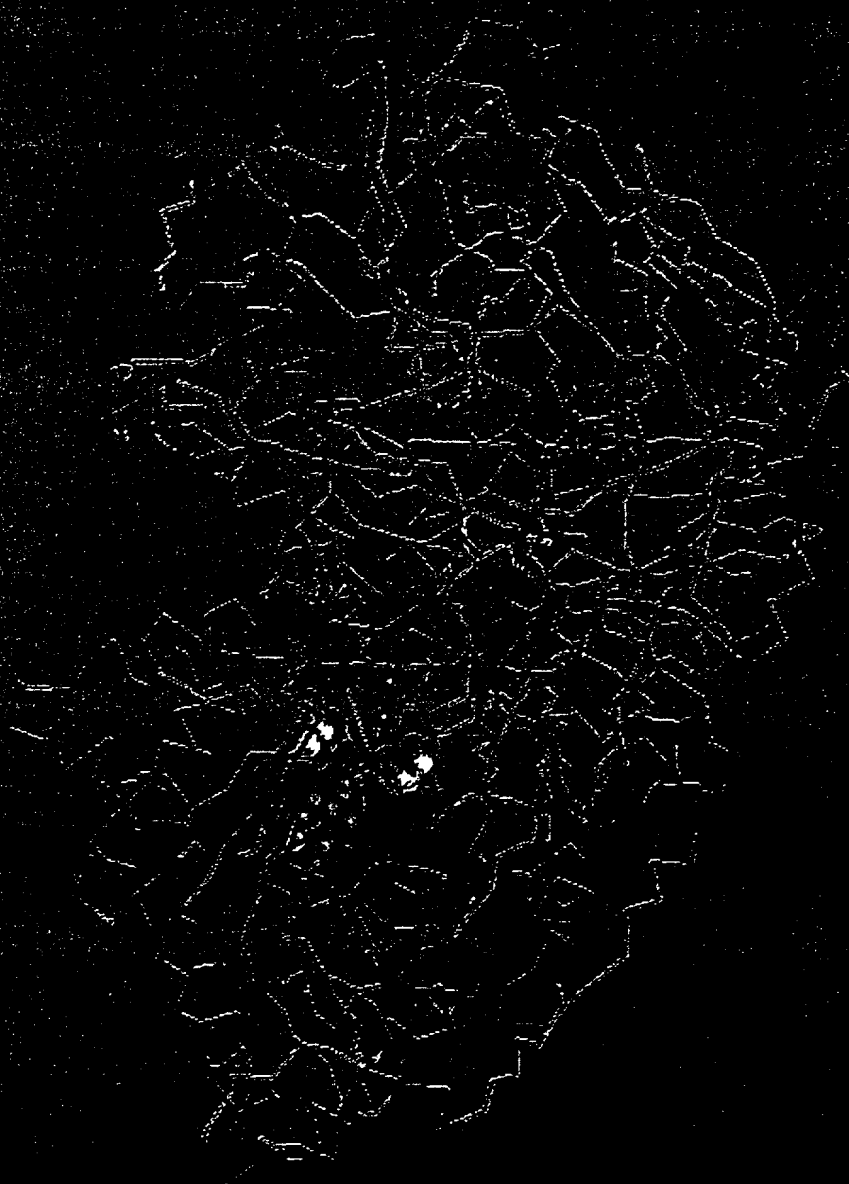


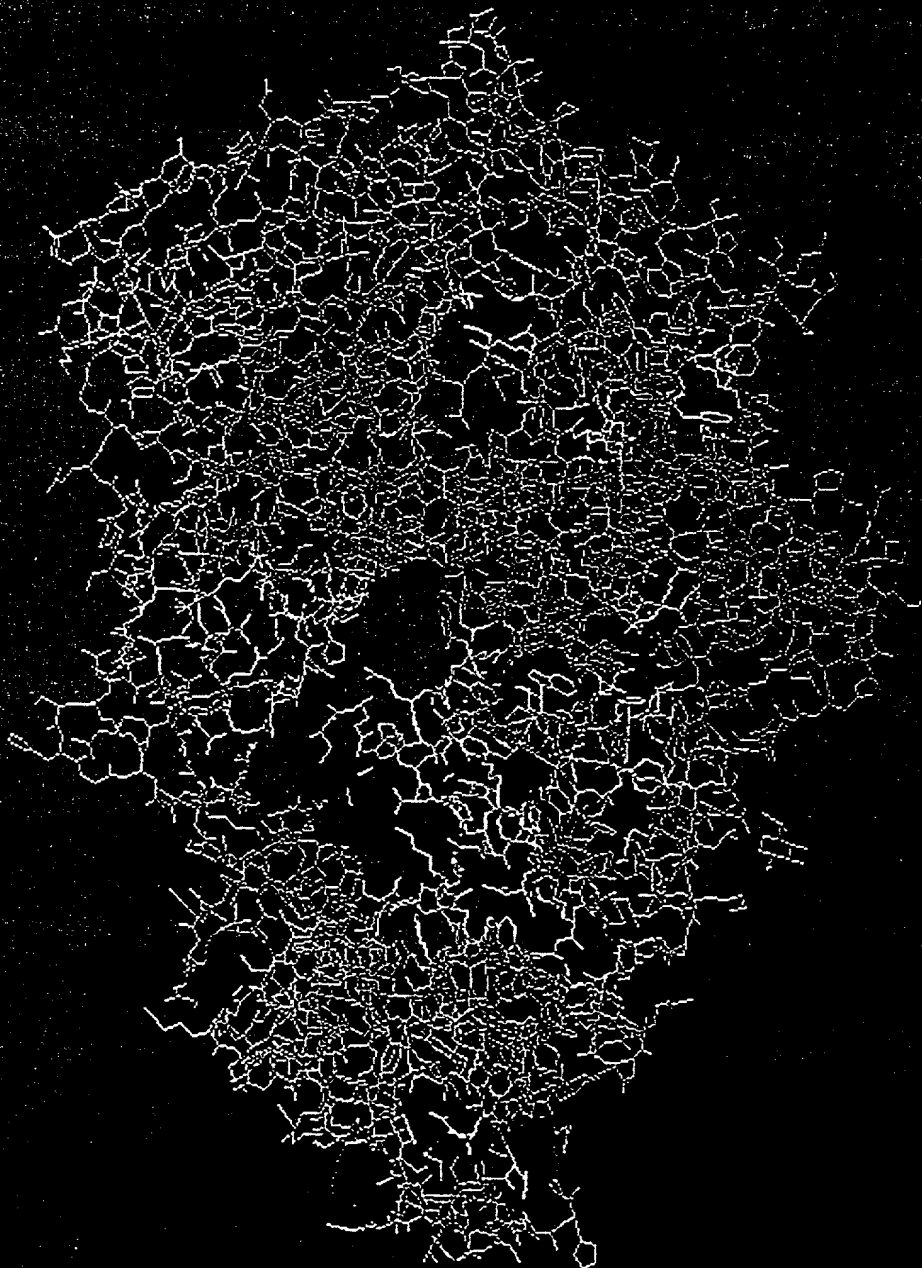
12243 19USU1



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12243.19USU1
MANNOSIDASE STRUCTURES
DOUGLAS P MUELLER
612 371 5237
SHEET 3 OF 20



Tris

1012260-96039600

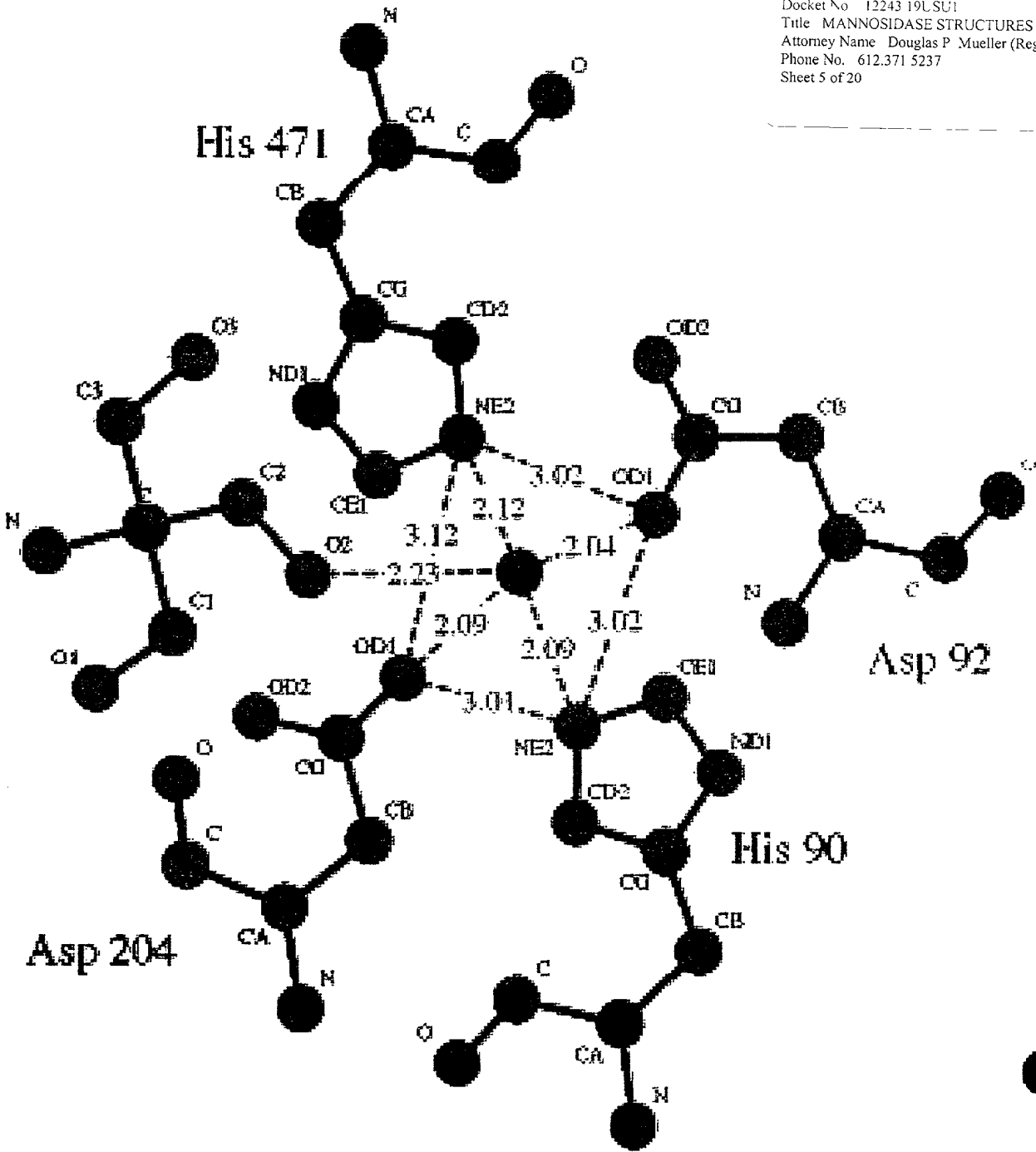
His 471

Asp 92

His 90

Asp 204

● Zn²⁺



pMT/BIp-N-HIS-dGMII [851 to 4042] -> 1-phase Translation

DNA sequence 6642 b.p. TCGCGGTTTCG ... AGGCCCTTTCGT circular

N-HIS added to pMTV5HISa (b/t BglII/EcoRI) for drosophila expression of tagged proteins.
Add drosophila dGMII from clone D11 (shortened 3') DAK/Tara 15-3-99

Inventor: ROSE et al.
Docket No.: 12243.19USU1
Title MANNOSIDASE STRUCTURES
Attorney Name Douglas P. Mueller (Reg. No. 30,300)
Phone No. 612 371 5237
Sheet 6 of 20

851/1	881/11	911/21
atg AAG TTA TGC ATA TTA CTG GCC GTC GTG GCC TTT GTT GGC CTC TCG CTC GGG aga tct agc cac cat cat cat cat cac gga gAA Ttc		
M K L C I L L A V V A F V G L S L G R S S H H H H H H H G E F		
941/31	971/41	1001/51
gac gat cca ata aga cct cca ctt aaa gtg gct cgt tcc ccg agg cca ggg caa tgc caa gat gtg gtc caa gac gtg ccc aat gtg gat		
D D P I R P P L K V A R S P R P G Q C Q D V V Q D V P N V D		
1031/61	1061/71	1091/81
gta cat atg ctg gag cta tac gat cgc atg tcc ttc aag gac ata gat gga ggc gtg tgg aaa cag ggc tgg aac att aag tac gat cca		
V Q M L E L Y D R M S F K D I D G G V W K Q G W N I K Y D P		
1121/91	1151/101	1181/111
ctg aag tac aac gcc cat cac aaa cta aaa gtc ttc gtt gtg ccg cac tgc cac aac gat cct gga tgg att cag acg ttt gag gaa tac		
L K Y N A H H K L K V F V V P H S H N D P G W I Q T F E E Y		
1211/121	1241/131	1271/141
tac cag cac gac acc aag cac atc ctg tcc aat gca cta cgg cat ctg cac gac aat ccc gag atg aag ttc atc tgg cgc gaa atc tcc		
Y Q H D T K H I L S N A L R H L H D N P E M K F I W A E I S		
1301/151	1331/161	1361/171
tac ttt gct cgg ttc tat cac gat ttg gga gag aac aaa aag ctg cag atg aag tcc att gta aag aat gga cag ttg gaa ttt gtg act		
Y F A R F Y H D L G E N K K L Q M K S I V K N G Q L E F V T		
1391/181	1421/191	1451/201
gga gga tgg gta atg ccg gac gag gcc aac tcc cac tgg cga aac gta ctg ctg cag ctg acc gaa ggg caa aca tgg ttg aag caa ttc		
G G W V M P D E A N S H W R N V L L Q L T E G Q T W L K Q F		
1481/211	1511/221	1541/231
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M N V T P T A S W A I D P F G H S P T M P Y I L Q K S G F K		
1571/241	1601/251	1631/261
aat atg ctt atc caa agg acg cac tat tgc gtt aag aag gaa ctg gcc caa cag cga cag ctt gag ttc ctg tgg cgc cag atc tgg gac		
N M L I Q R T H Y S V K K E L A Q Q R Q L E F L W R Q I W D		
1661/271	1691/281	1721/291
aac aaa ggg gac aca gct ctc ttc acc cac atg atg ccc ttc tac tgc tac gac att cct cat acc tgt ggt cca gat ccc aag gtt tgc		
N K G D T A L F T H M M P P F Y S Y D I P H T C G P D P K V C		
1751/301	1781/311	1811/321
tgt cag ttc gat ttc aaa cga atg ggc tcc ttc ggt ttg agt tgt cca tgg aag gtg ccg ccg cgt aca atc agt gat caa aat gtg gca		
C Q F D F K R M G S F G L S C P W K V P P R T I S D Q N V A		
1841/331	1871/341	1901/351
gca cgc tca gat ctg ctg gtt gat cag tgg aag aag aag gcc gag ctg tat cgc aca aac gtg ctg ctg att ccg ttg ggt gac gac ttc		
A R S D L L V D Q W K K K A E L Y R T N V L L I P L G D D F		
1931/361	1961/371	1991/381
cgc ttc aag cag aac acc gag tgg gat gtg cag cgc gtg aac tac gaa agg ctg ttc gaa cac atc aac agc cag gcc cac ttc aat gtc		
R F K Q N T E W D V Q R V N Y E R L F E H I N S Q A H F N V		
2021/391	2051/401	2081/411
cag cgc cag ttc ggc aca ctg cag gaa tac ttt gat gca gtg cac cag gcg gaa agg gcg gga caa gcc gag ttt ccc acg cta agc ggt		
Q A Q F G T L Q E Y F D A V H Q A E R A G Q A E F P T L S G		
2111/421	2141/431	2171/441
gac ttt ttc aca tac gcc gat cga tgc gat aac tat tgg agt ggc tac tac aca tcc cgc ccg tat cat aag cgc atg gac cgc gtc ctg		
D F F T Y A D R S D N Y W S G Y Y T S R P Y H K R M D R V L		
2201/451	2231/461	2261/471
atg cgc tat gta cgt gca gca gaa atg ctt tcc gcc tgg cac tcc tgg gac ggt atg gcc cgc atc gag gaa cgt ctg gag cag gcc cgc		
M H Y V R A A E M L S A W H S W D G M A R I E E R L E Q A R		
2291/481	2321/491	2351/501
agg gag ctg tca ttg ttc cag cac cac gac ggt ata act ggc aca gca aaa acg cac gta gtc gtc gac tac gag caa cgc atg cag gaa		
R E L S L F Q H H D G I T G T A K T H V V V D Y E Q R M Q E		
2381/511	2411/521	2441/531
gct tta aaa gcc tgt caa atg gta atg caa cag tgc gtc tac cga ttg ctg aca aag ccc tcc atc tac agt ccg gac ttc agt ttc tgc		
A L K A C Q M V M Q Q S V Y R L L T K P S I Y S P D F S F S		
2471/541	2501/551	2531/561
tac ttt acg ctc gac gac tcc cgc tgg cca gga tct ggt gtg gag gac agt cga acc acc ata ata ctg ggc gag gat ata ctg ccc tcc		
Y F T L D D S R W P G S G V E D S R T T I I L G E D I L P S		
2561/571	2591/581	2621/591
aag cat gtg gtg atg cac aac acc ctg ccc cac tgg cgg gag cag ctg gtg gac ttt tat gta tcc agt ccg ttt gta agc gtt acc gac		
K H V V M H N T L P H W R E Q L V D F Y V S S P F V S V T D		
2651/601	2681/611	2711/621
ttg gca aac aat ccg gtg gag gct cag gtg tcc ccg gtg tgg agc tgg cac cac gac aca ctc aca aag act atc cac cca caa ggc tcc		
L A N N P V E A Q V S P V W S W H H D T L T K T I H P Q G S		
2741/631	2771/641	2801/651
acc acc aag tac cgc atc atc ttc aag gct cgg gtg ccg ccc atg ggc ttg gcc acc tac gtt tta acc atc tcc gat tcc aag cca gag		
T T K Y R I I F K A R V P P M G L A T Y V L T I S D S K P E		
2831/661	2861/671	2891/681
cac acc tgc tat gca tgc aat ctc ttg ctc cgt aaa aac ccg act tgc tta cca ttg ggc caa tat ccg gag gat gtg aag ttt ggc gat		
H T S Y A S N L L L R K N P T S L P L G Q Y P E D V K F G D		
2921/691	2951/701	2981/711
cct cga gag atc tca ttg cgg gtt ggt aac gga ccc acc ttg gcc ttt tgc gag cag ggt ctc ctt aag tcc att cag ctt act cag gat		
P R E I S L R V G N G P T L A F S E Q G G L L K S I Q L T Q D		
3011/721	3041/731	3071/741
agc cca cat gta ccg gtg cac ttc aag ttc ctc aag tat ggc gtt cga tgc cat ggc gat aga tcc ggt gcc tat ctg ttc ctg ccc aat		
S P H V P V H F K F L K Y G V R S H G D R S G A Y L F L P N		
3101/751	3131/761	3161/771
gga cca gct tgc cca gtc gag ctt ggc cag cca ctg gtc ctg gtg act aag ggc aaa ctg gag tgc tcc gtc agc gtg gga ctt ccg agc		
G P A S P V E L G Q P V V L V T K G K L E S S V S V G L P S		
3191/781	3221/791	3251/801
gtg gtg cac cag acg ata atg cgc ggt ggt gca cct gag att cgc aat ctg gtg gat ata ggc tca ctg gac aac acg gag atc gtg atg		
V V H Q T I M R G G A P E I R N L V D I G S L D N T E I V M		
3281/811	3311/821	3341/831
cgc ttg gag acg cat atc gac agc ggc GAT ATC ttc tac acg gat ctc aat gga ttg caa ttt atc aag agg cgg cgt ttg gac aaa tta		
R L E T H I D S G D I F Y T D L N G L Q F I K R R R L D K L		

Figure 7

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ALIGNMENT OF EXPRESSED SECRETED DROSPHILA MANNOSIDASE
WITH HUMAN MANNOSIDASE
Percent Similarity: 52.157 Percent Identity: 43.039

1RSSHHHHHGFED 13
51 LOEKIDHLERLAENNEIISNIRDSVINSESVEDGPKSSQSNFSQAGS 100
14 DPIRPLKVARSPRGQODVVQD.VPNVDVQMLELYDRMSFKDIDGGW 62
101 HLL..PSQLSVDTADCLFASQSGSHNSDVQMLDVYSLISFSDNPDGGW 148
63 KOGWNIKYDPLKYNAAHKLKVFVPHSHNDPGWITQTEEXYQHDPKHLS 112
149 KOGFDIYESNEWDT.EPLQVFPVPHSHNDPGWLKTDFNFRDKTYIFN 197
113 NALRHLHNDPEMKFIWAEISYFARFVHDLGENKKLQMKSIKNGOLEFVT 162
198 NMVLKUKEDSRKFIWSEISYLSKWDIIDIQKDAVKSLIENGQLEIVT 247
163 GGMVMDPEANSHWRNVLQLTEGQWLKQFANVTPTASWAIDPFGHSPM 212
248 GGMVMDPEATHYFALIDQLECHQWLENNIGVKPRSGMALDPFGHSPM 297
213 PYILOKSGFNMLIQRTHYSVKKELAAQQLQLEFLWRQIWNKGDALFTH 262
298 AVLNRAGLSHMLIQRVHYAVKKFALHKTLEEFWRQWDLGSVTDILCH 347
263 MNPFSYDIPTHTCGPDPKVCQCFDKRMGSFGLSCPWKVPPRTISDQNV 312
348 MNPFSYDIPTHTCGPDKICQCFDKRLPGRGCPWGVPEPETHPGNVQ 397
313 ARSDDLVDQWKKAEALRTNVLILPLGDDFRKQNTENDVQRVNYERLF 362
398 SRARMLDQYRKSKLFRTKVLALPLGDDFRYCEYTEMWDLQFKNYQLFD 447
363 HINQAHFNVAQFGTQYEDAVHQAERA...QOAEFFPLSGDFFTYA 408
448 YNNSQSKFKVKIQGTLSDFDQALDKADETQDRKQSMFPVLSGDFFTYA 497
409 DRSDNTWSGYTSPYHKRMDRVLNHYVPAEMLSAW.....HSWD.... 449
498 DRDDHYWSGYTSPFYKRMDRIMESHFAAEILYFALRQAHKYKINKF 547
450 GNARIEERLQARELSLFQHHDGITGTAHVVDYEQRMQAEALKACQM 499
548 LSSSLYALTAEARNLGLFQHHDAITGTAKDWVVDYGTRLFHSLMWLEK 597
500 VMQQSIVRLITKPSIYSPDFSFYFTLDDSRWPGSGVEDSRTTILGEDI 549
598 IIGNSAFLIGDKLTDVSDPTFLEMDLKQKQSDSLPQKNIIIRLSAE. 646
550 LPSKHVVMHTLPHWRBQLVDYVSSPFVSVDTLANNPVEAQVSPVMSWH 599
647 ..PRVLVNVNPLEQDRISLVSVYSSPTQVFSASGKPEVQVSAVW... 691
600 HDTLAKTHPQSGTTKYRIIFKARVPPMGLATVLTISDSKPEHTSVASN 649
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650 LLLRKNPTSLPLGQYEPEDVAFGDPREISLRVGNCGPTLAFSEQGLKLSIQL 699
733 VLYKXKVE.DSGIFTIKNMINTEGITLE.NSFVLLRFDTGLMKQNT 780
700 TQDSPHVPVHFELKYGVRSRGDRSGAYLFLPENGASP.VELGQPVVLVT 748
781 KEDGKHHEVNVQFSWYGTTIKRDKSGAYLFLPDGNAPVYVYTPFPFVAVT 830

Monday August 21, 2000

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749 KGKLESSVSVGLPSVVHQTIM.....RGGAPEIRNLVDIGSLDNTIEVM 792
831 HGRIYSEVTCTFFDHVTHRVRLYHIQIEGQSVESVNIVDIRKVVNREIAM 880
793 RLETHIDSGDIFVTDNLGLQFIKRRRLDKPLQANYYPPIPSGMFIEDANT 842
881 KISSDIKSONRFFYDNLGYQIQPRMTLSKLPLQANYVPMTTMAYIQDAKH 930
843 RLTLTGTGPIGSSSLASGELEIMODRRRLASDDBERGLGQGVLDNKPVLHIY 892
931 RLTLLSAQSLSGSSSLNSGQIEVIMDRRLMQDDNRGLEQGIQDNKITANLF 980
893 RLVLKVNANCVRPSKLHPAGVLTSAAHKASQSLLDPL.....DKTFAENE 938
981 RILLEKRSANVTEEEKSVSYPSLLSHITSSLMNHVPIPMANKF...SSP 1027
939 WIGAQQGQGGDHPASAREDLDVSVMRRLTK..SSAKTORVGVVLRHTNLMQ 986
1028 TLEQGEFSPLQSSSLPCDIHLVNLRTIOSKVGNGHSEAAALILHRKG.FD 1076
987 C.....GTPÉ...EHTQ.KLDVCHLLPN..VARCETTLTFLQNLHLDCM 1026
1077 CRFSSKGTGLFCSTQOKILVQKLLNKFIVESLTPSSLSLMHSPPGTONI 1126
1027 VAPEVCPMETAAYVSSHSS 1045
1127 SEINLSPMEISTFRIQLR. 1144

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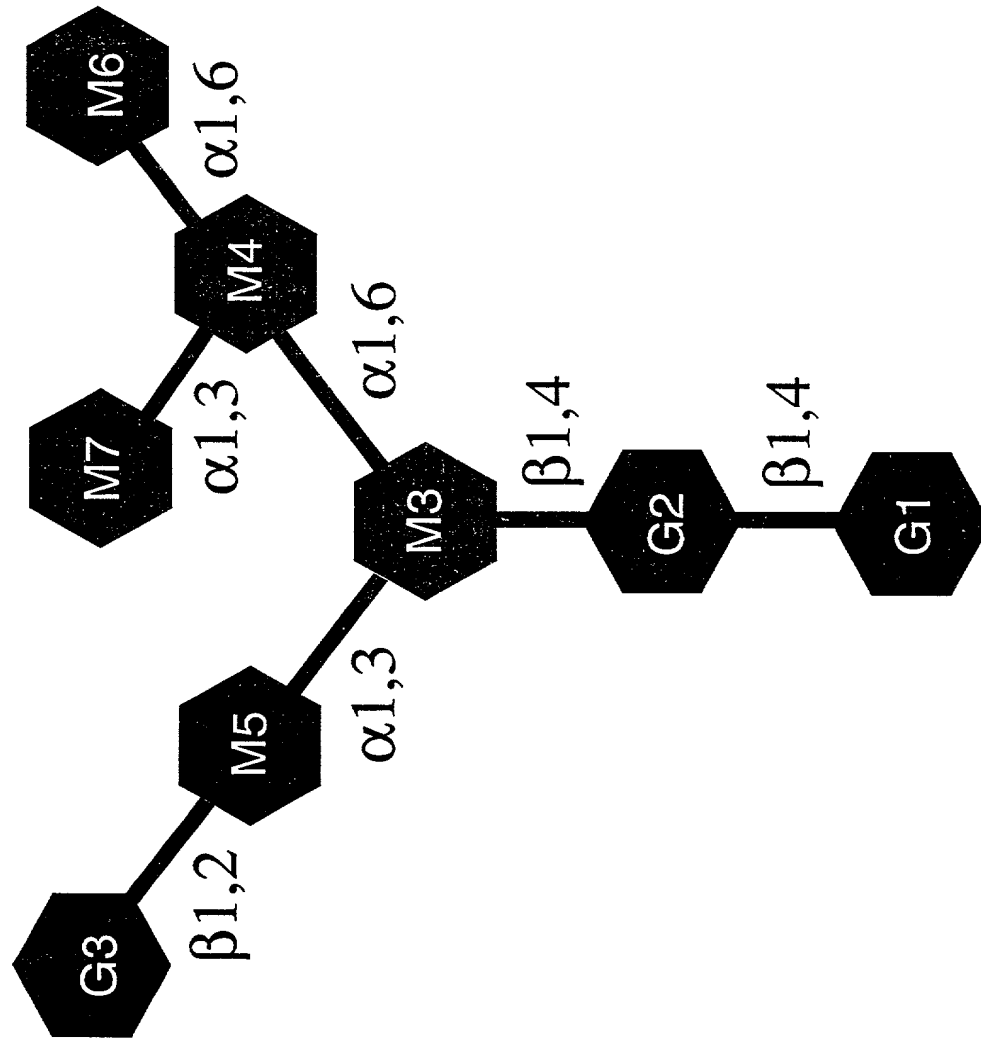
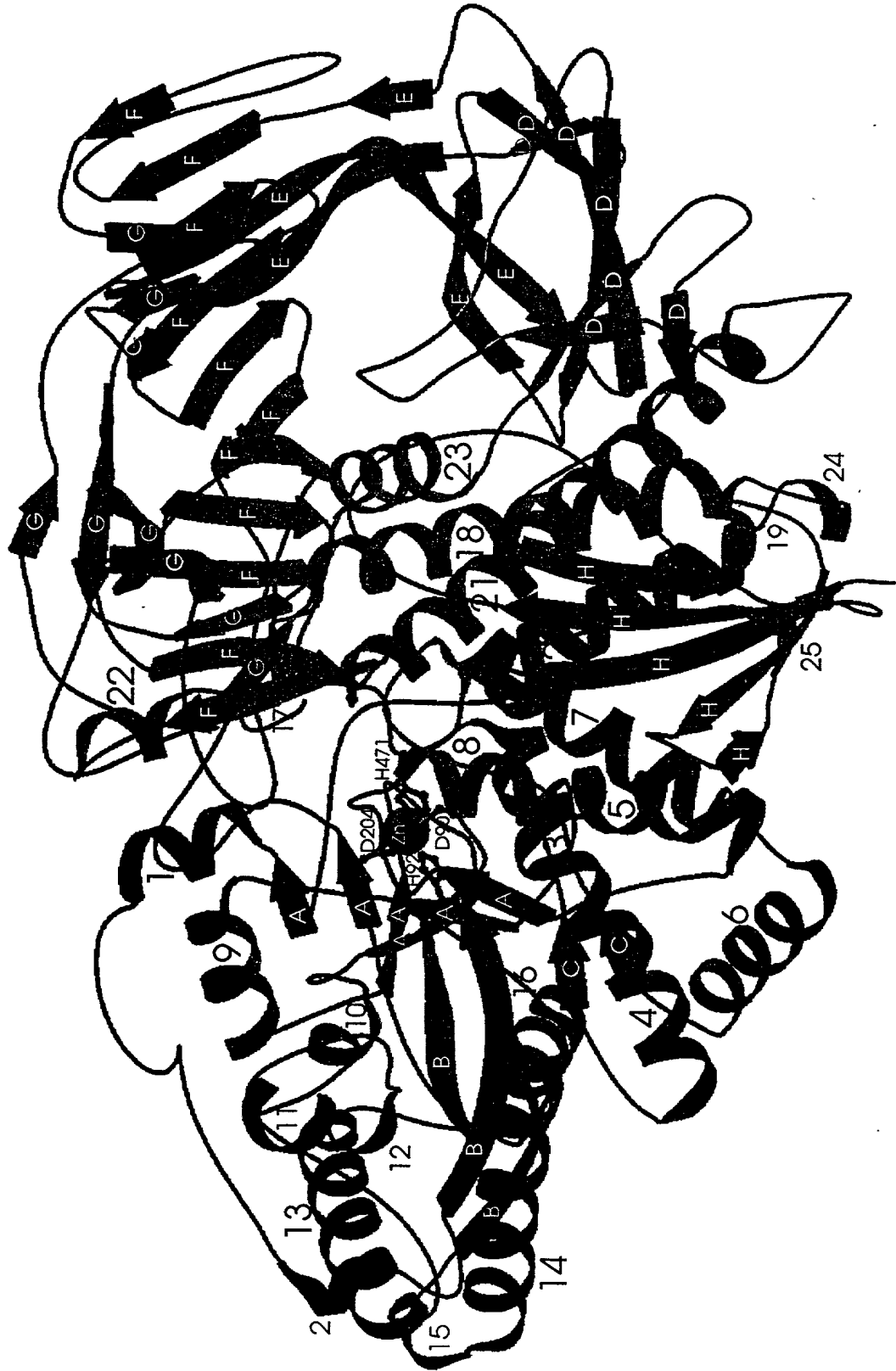


Figure 8B



111-220-920-960

Figure 8C



Fig. 9A



Fig. 9B

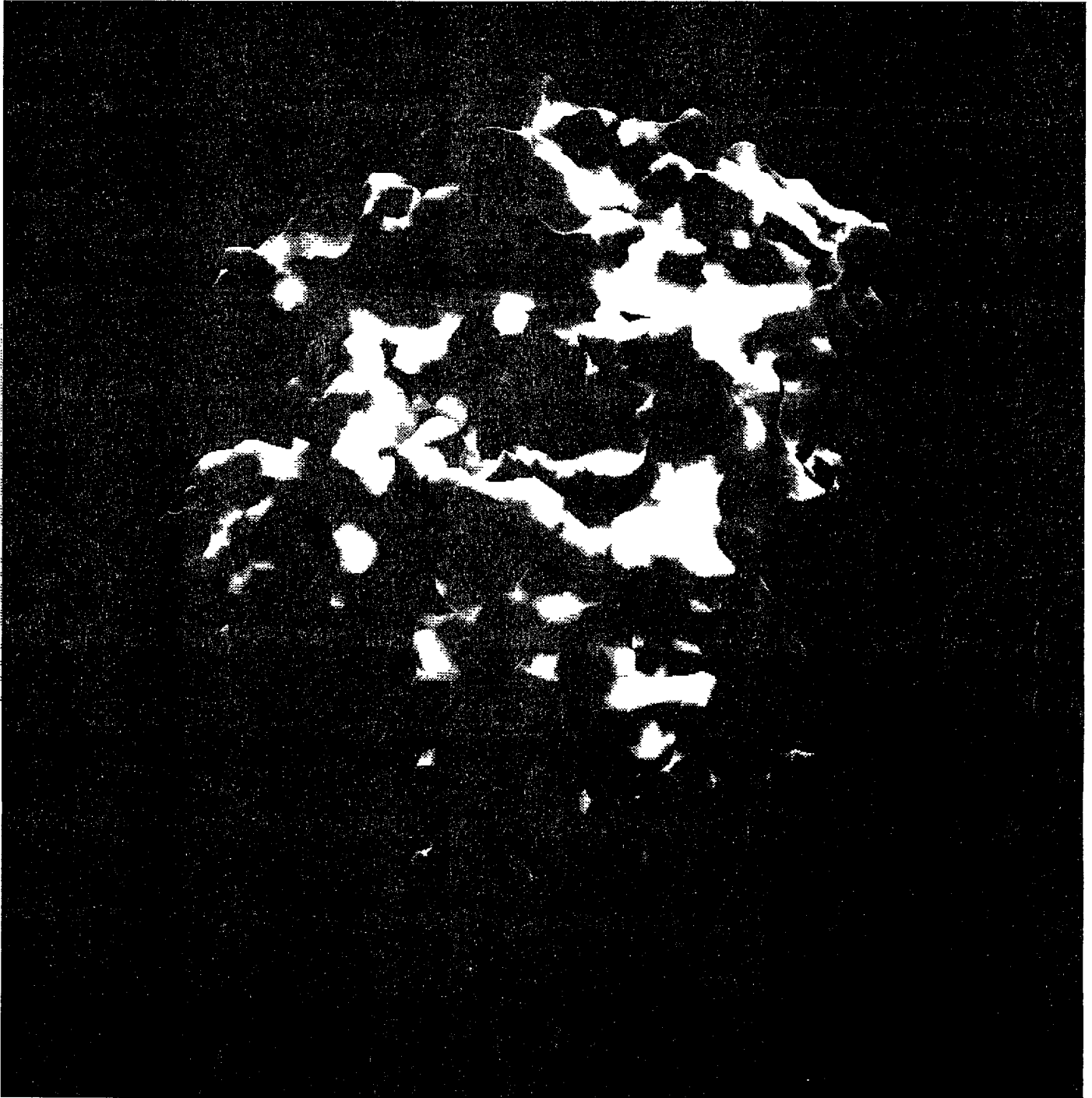


Fig. 9C

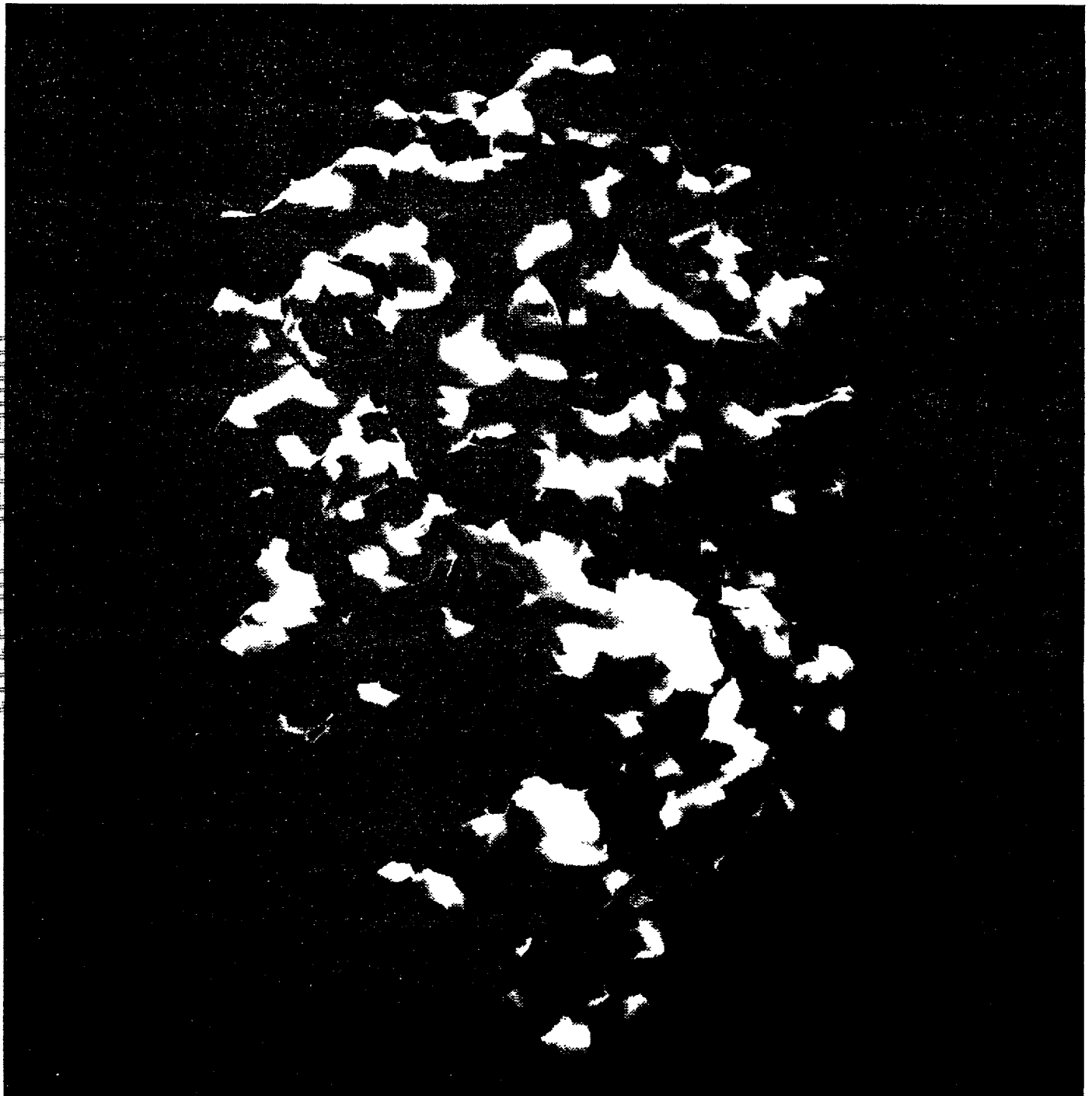


Figure 10B

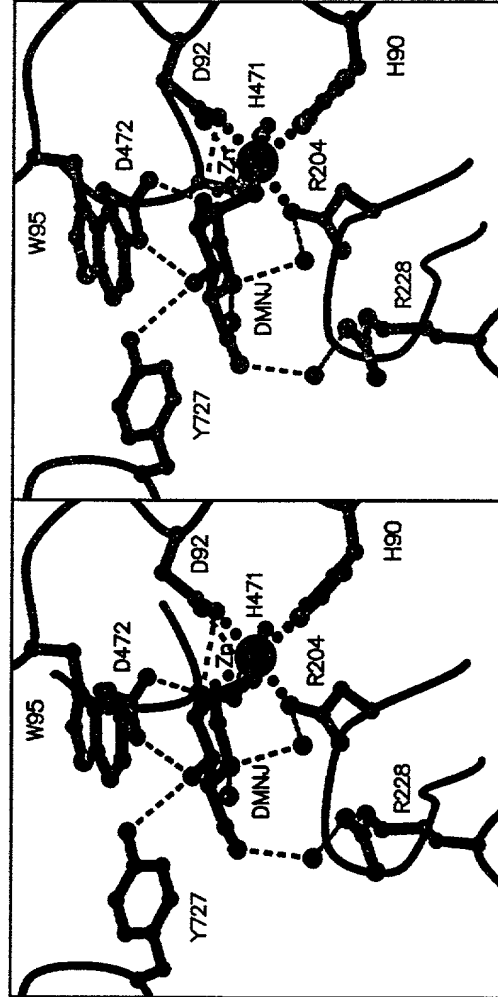


Figure 10C

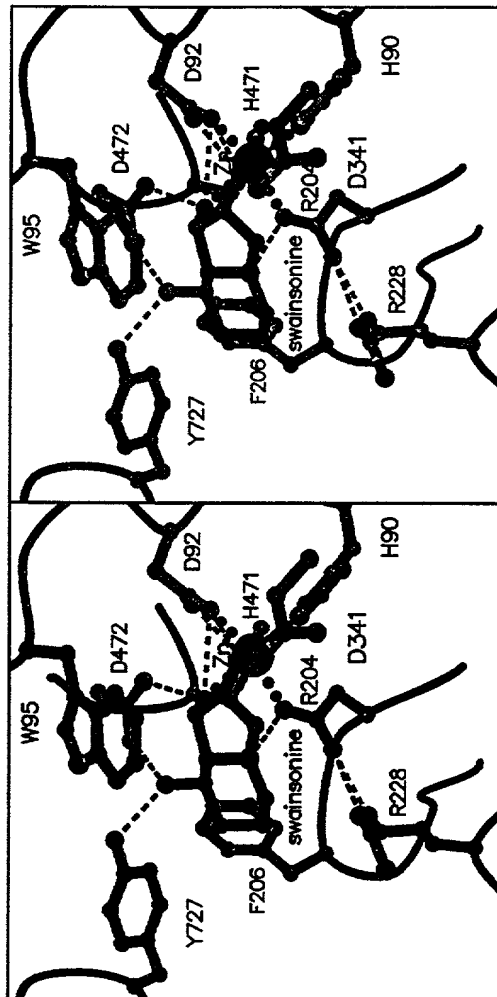


Figure 11A

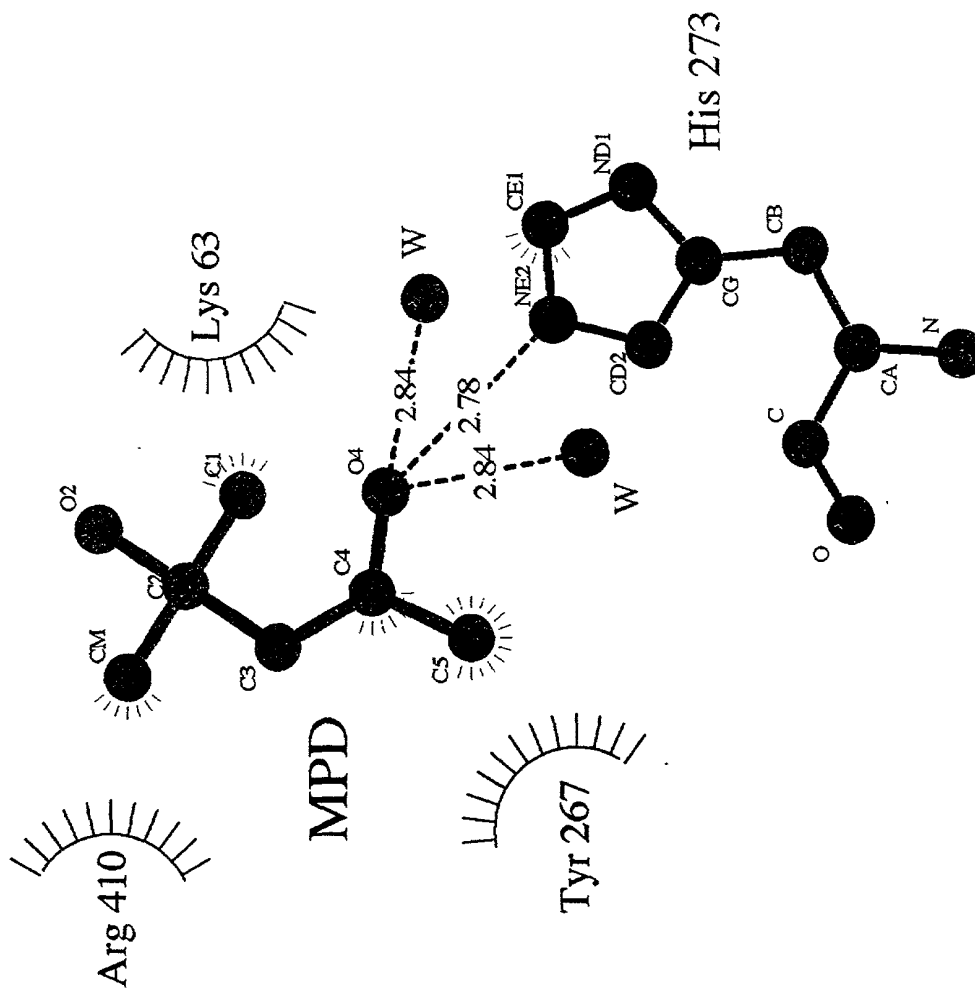




Fig. 11B

Figure 11C

